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(54) Title: CARRIER SYSTEMS FOR DRUGS

(57) Abstract

The invention relates to carrier systems for drugs, their preparation and their use. The carrier systems according to the invention exhibit spherical particles with a diameter of less than 1 µm, optionally in combination with an appropriate bioadhesive polymer. The carrier systems have an improved bioadhesiveness and a high loadability with drugs and are able to provide a stable, pharmaceutically active concentration of drugs at the site of action over a longer period of time.

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Carrier Systems for Drugs

The invention relates to carrier systems for drugs, their preparation and their use.

The therapeutic effect of a drug inter alia is dependent on the concentration of the drug at the site of action for a desired period of time. On grounds of this dependence, factors such as distribution, dilution, excretion, absorption or biotransformation play an important role for the therapeutic effect of a drug. All of these factors must be taken into account in particular when formulating a drug.

One possibility of improving the therapeutic effect of a drug is to use carrier systems such as viscous solutions, ointments, bioadhesive polymers or spherical particles [1-9]. U.S. Patent 4,617,186 discloses, for instance, a cationic polymer ("GAFQUAT-234") that possesses bioadhesive properties and can be used as a carrier system for drugs for the treatment of eye diseases; Moreover, this polymer is also said to be able to bind spherical particles of albumin, which also represent carrier systems for drugs. The complexes of the polymer and the carrier system are said to be bioadhesive and to retard the drug release but no comparative data vis-à-vis the polymer alone are given in support of this statement. Furthermore, in particular cationic polymers are to be considered problematic because of their toxicological properties.

Solutions, ointments and specific polymers distinguish themselves in particular by their high capacity for drug incorporation. Solutions exhibit considerable disadvantages over ointments and polymers due to the fast dilution, excretion and biotransformation of the drug, which entails that the drug concentration drops rapidly below the pharmaceutically active level at the site of action. Ointments, when applied to the eye, lead for example to a severe impairment of vision. A disadvantage of the known spherical particles as carrier systems is above all the low drug incorporation capacity, which may also entail too low a concentration of a drug at the site of action. Another disadvantage of known spherical particles is their low bioadhesiveness, which leads to a rapid excretion of these particles.

The problem underlying the invention is the provision of carrier systems for drugs, which remain for a prolonged time at the site of application by an improved bioadhesiveness, exhibit a high loadability with drugs and provide a stable concentration of drugs at the site of action over the desired period of time, in order to improve the therapeutic effect of drugs.

This problem is solved by the features of the claims.

In a first embodiment, the carrier system of the present invention exhibits spherical particles with a diameter of less than 1 µm, preferably less than 500 nm, most preferably 100 nm to 300 nm. In the following, such particles will also be called nanoparticles. By "particle size" the mean diameter of the particles is meant.

Nanoparticles as a carrier system for drugs display various advantages ver the known microparticles with a diameter f at least 1 µm. The nanoparticles can be better distributed in a liquid since n significant sedimentation of the

particles takes place. As a rule, no surfactants need to be added in order to disperse the particles. The nanoparticles can also be used as drug vehicles in inhalation aerosols. The nanoparticles have a larger specific surface and thus a higher incorporation capacity. Thus they enable an enhanced effect of the drug when used as a carrier system.

The spherical particles of the present invention preferably contain at least one synthetic, semi-synthetic and/or natural biopolymer, most preferably a polypeptide such as albumin or gelatine. Functional groups of the biopolymer such as $-NH_2$, $-CO_2H$, -COH or -SH permit covalent bonds with a multitude of drugs.

The spherical particles according to the present invention can incorporate both hydrophobic and hydrophilic drugs, wherein the loadability generally depends on the drug, e.g. 15 % by weight of pilocarpine with respect to the spherical particles, and the weight ratio of particle to drug can be up to 1:1.

The spherical particles are non-toxic, biodegradable by lysosomal enzymes, biocompatible, physically and chemically stable and do not possess any relevant antigenic properties.

Furthermore, the spherical particles of the invention have a controllable drug release rate and are rapidly excreted.

Another embodiment of the carrier system according to the present invention comprises spherical particles with a diameter of at least 1 nm and less than 1 mm, i.e. microparticles and nanoparticles, in combination with at least one bioadhesive polymer such as pectins (p lygalactur nic acid), mucop lysaccharides (hyalur nic acid, mucin) or non-toxic lectines. In the following, such a carrier system will also be called particle/polymer carrier system. Not all bioadhesive polymers known in the state of

the art necessarily entail a synergetic effect when used as a carrier system in connection with spherical particles. The use of polysaccharides, polyacrylates, alginates, polyvinyl alcohol, polyethylene glycol, polyvinyl pyrrolidone and lectines is preferred. Most preferred is the use of methyl cellulose 400, sodium carboxymethyl cellulose, Carbopol® 941, hydroxypropyl methyl cellulose, hyaluronic acid, sodium alginate MV, mucin and polycarbophil.

The bioadhesive polymers preferably have a viscosity of 4 x 10^{-3} to 100 x 10^{-3} Pas, the retarded drug release being improved at a higher viscosity. Generally, a higher viscosity of the polymers is advantageous. However, the viscosity increase is restricted for practical reasons, for example in the application to the eye. The weight ratio of spherical particles to bioadhesive polymer inter alia is dependent on the used polymer and can for instance be 2:1 to 1:2.

The advantages of particle/polymer carrier systems for drugs over pure particle carrier systems are on the one hand an increased incorporation capacity due to an increased adsorption of the drug molecules and on the other hand a lower required dose of the drug due to a prolonged effect of the drug and thus less discomfort for the patient.

The bioadhesive effect of the polymers is probably due to an intermolecular interaction, such as ionic interactions, Van der Waals interactions, hydrogen bonds or molecular entanglement of the polymer with surface components, such as proteins or lipids, of mucous surfaces, or to other physical phenomena, such as capillary action or viscosity.

A further aspect of the invention is a compositi n that c ntains at least on of the aforementi ned carrier systems, a drug and optionally a further pharmaceutically acceptable carrier or diluent.

The weight ration of drug to carrier system is conventionally in the range of 100:1 to 1:1000, preferably 10:1 to 1:10 and most preferably 2:1 to 1:2 or 2:1 to 1:1.

The preparation of the spherical particles according to the invention can be carried out by several alternative methods. Suitable methods are the desolvation of the biopolymer used as starting material by dehydrating compounds, such as alcohols or sodium/ammonium sulfate, the thermal denaturation of the biopolymer by heating to 95°C to 195°C, the reaction of the biopolymer with a coupling reagent and/or the reaction of the biopolymer with a compound ("hardener") having two or more functional groups, such as glutaraldehyde.

The resultant spherical particles are suspended in a concentration of up to 10 % (w/v) in an appropriate solvent, for instance water.

The size as well as the diameter of the spherical particles can be optimized by varying appropriate parameters, such as temperature, concentration of the biopolymer, concentration of the hardener or selection of the dehydrating agent (e.g. absolute alcohol instead of salts), or by further appropriate method steps, such as ultrasonication of the particles. Furthermore, the spherical particles can also be chromatographically purified over a suitable column (gel filtration).

A preferred method for the preparation of the spherical particles comprises the addition of 100 % ethanol to a solution of 0.25 to 1.5 % (w/v) of a polypeptide, preferably less than 1.25 % (w/v) of the polypeptid , in distilled water, the mixing rati of ethan l:polypeptide solution being > 1:1 to 2:1. After the onset of the polypeptide des lvation, 0.01 to 1 % (v/v) of 25 % glutaraldehyde are

added to said mixture. After about 1 hour, a corresponding amount of a 12 % (w/v) sodium metabisulfite solution is added in order to decompose the excess glutaraldehyde. After about 3 hours, the ethanol is evaporated and the obtained particle suspension column-chromatographically purified. The particle-containing fraction is subsequently lyophilized while glucose is added.

When preparing the spherical particles, intermolecular and intramolecular bonds, such as covalent bonds, or interactions, such as hydrophobic interaction, with particular functional groups of the biopolymer, such as $-NH_2$, $-CO_2H$, -COH, -SH or phenyl groups, are produced.

The preparation of the particle/polymer carrier systems according to the invention comprises mixing at least one appropriate bioadhesive polymer with a suspension of spherical particles. Said spherical particles can be produced according to the aforementioned inventive method or according to methods known in the state of the art [10-12].

The preparation of the composition of a drug and a carrier system according to the invention comprises the adsorption or loading of a drug into or onto the spherical particle and can be performed either simultaneously with the preparation of the carrier system by the addition of an appropriate drug solution or sequentially by the addition of a suspension of spherical particles to an appropriate drug solution. Furthermore, the preparation optionally comprises the addition of 0.1 to 2 % of a surfactant.

The loading process of the carrier system with a drug is probably based on a bond of the drug molecules with the carrier system, in which said molecules are complexed by intermolecular interactions, such as hydrogen bonds, with specific groups of the biopolymer, such as $-NH_2$, -OH, -COOH or -SH.

The carrier system according to the invention can incorporate a multitude of drugs, such as antiasthmatics, analgetics, antitussiva, bronchodilators, narcotics, mucolytics, antibacterials, antifungals, antituberculosis agents, steroids, antitumor agents, parasympathomimetics, fibrinolytics, immunosuppressives etc.

The drug-loaded carrier systems according to the present invention can be administered intraarticularly, cutaneously, subcutaneously, intramuscularly, intravenously, intraarterially, intravaginally, rectally, orally, nasally and ocularly. The drug-loaded particle/polymer carrier systems are preferably applied onto mucous surfaces of mammalians, including humans.

A preferred application comprises the formulation of a composition of carrier systems and drugs, which are administered for the treatment of eye diseases, such as glaucoma, inflammations, infections and allergic reactions.

When selecting the particle size, the intended application plays an important role. For example, carrier systems that contain spherical particles with a diameter of more than 25 µm are not suitable for the application to the eye because of the pain sensation. The lowest limit for the particle size is essentially not restricted by the application, however, it is difficult to produce particles with a diameter of < 10 nm. Furthermore, particles with a diameter of < 10 nm lead to a rapid accumulation at the eye or to an exhalation in the application as an inhalation aerosol.

In the following the invention will be explained in more detail by means of the drawings.

Figure 1 shows a diagram of the miotic activity of a pilocarpine composition containing albumin nanoparticles against time, with a 2 % pilocarpine solution as a reference.

Figure 2 shows a diagram of the miotic activity of a nanoparticle/mucin/pilocarpine composition (weight ratio 1:1.25:1) against time, with a mucin/ pilocarpine composition (weight ratio 1.25:1) as a reference.

Figures 3 and 4 show a diagram of the intraocular pressure (mm Hg) of a 2 % pilocarpine solution, a microparticle/ pilocarpine composition and a nanoparticle/mucin/pilocarpine composition against time, wherein the temporal change of the pressure without the addition of a drug is defined as a baseline.

The following examples illustrate the invention.

Example 1: Albumin Nanoparticle/Pilocarpine Composition

(A) The Preparation of the Albumin Nanoparticles

500 mg bovine serum albumin are dissolved in 40 ml of distilled water and 100 % ethanol is slowly dropped in while stirring is maintained. After the addition of about 60 ml of 100% ethanol, the desolvation of the bovine serum albumin can be observed by a slight blue shimmer of the mixture. 0.1 ml of 25 % glutaraldehyde is added to the mixture during stirring and subsequently agitation is continued for about 3 hours. The excess glutaraldehyde is decomposed by the addition of 1 ml of 12 % sodium metabisulfite solution. After further 3 hours of agitation, the ethanol is evaporated under vacuum. The remainder is chromatographically purified over a Sephacryl S-1000 column (Pharmacia). The obtained particle suspension is lyophilized by the addition of

glucose for about 16 hours. The particle diameter in this method is 100 to 200 nm (measured with particle measuring device BI-90, Brook Haven Instruments).

(B) Pilocarpine-Loading

20 mg/ml of the nanoparticles are added to a 2 % pilocarpine nitrate solution (containing 1.2 % of pluronic F 68, 1 % of sodium sulfate, phosphate-buffered, pH 7) and the mixture is equilibrated to reach an equilibrium while stirring. The mixture is then filtrated by ultrafiltration and the amount of the free pilocarpine is spectroscopically determined. The amount of incorporated pilocarpine is 11.8 mg/100 mg carrier. The amount of incorporated pilocarpine in the case of particles with a diameter of 1-2 µm is only 5.8 mg/100 mg carrier.

(C) Determination of the Miotic Activity

The determination of the miotic activity is carried out with male albino New Zealand rabbits. Each of the experiments is performed with 5 rabbits and a dose of 50 µl of nanoparticle/pilocarpine composition. The measurements of the pupillary diameter are carried out under constant light conditions with a micrometer that is held at a fixed distance from the rabbit's eyes. The results are graphically depicted in Figure 1. The duration of effect of pilocarpine increases by up to 14 %, with the half-life (t 1/2) being prolonged by up to 19 %. The half-life is defined as the moment at which the miosis exhibits half of its maximum value.

Example 2: Nanoparticle/Mucin/Pilocarpine Composition

(A) The Preparation of the Bovine Serum Albumin/Mucin Composition

The nanoparticles as described in example 1(A) are suspended in an appropriate buffer, pH 7, and 2.5 % or 4.5 % of mucin are added, solutions with viscosities of 4-7 x 10^{-3} Pas or 13-17 x 10^{-3} Pas, respectively, being obtained.

(B) Pilocarpine-Loading

The nanoparticle composition is suspended in a 2 % pilocarpine solution as described in example 1(B), and subsequently mucin is added.

(C) Determination of the Miotic Activity

The determination of the miotic activity is carried out as described in example 1(C). The results are graphically depicted in Figure 2 and in Table I. The effect of pilocarpine (Pilo.) is prolonged by up to 90 min (duration of effect [min]), the half-life (t 1/2) being prolonged by up to 62 %. The effect of pilocarpine is directly proportional to the miosis.

TABLE I

Carrier System	Measuring time[a] [min]	I max[b] [mm]	Duration [min]	AUC[c]	t1/2 [min]
Mucin 2.5% Micro-particle 2% Pilo. 2%	30	2.66	300	386.40	130
Mucin 2.5% Pilo. 2% Reference	30	2.40	210	230.74	97
Mucin 2.5% Nano-particle 2% Pilo. 2%	15	4.26	300	631.95	155
Mucin 2.5% Pilo. 2% Reference	30	3.68	210	394.53	122
Mucin 4.5% Micro-particle 4% Pilo. 2%	30	3.08	300	425.25	135
Mucin 4.5% Nano-particle 4% Pilo. 2%	30	4.15	300	629.74	157

a) moment of maximum pupillary contraction
b) maximum pupillary contraction
c) "area under the curve" (integral of the time-of-effect curve)

(D) Determination of the Intraocular Pressure (Betamethasone Model)

0.8 ml of betamethasone is subconjunctivally injected into the right eye of 13 male albino New Zealand rabbits. The injections are performed weekly over a period of 3 weeks. After three weeks, the ocular hypertension becomes stable. 50 µl of a particle/pilocarpine composition or a particle/mucin/pilocarpine comp sition are subsequently instilled into th conjunctival sac and then the intra cular pressure is measured. The results are graphically depicted in Figures 3 and 4 as well as in Table II. The time-of-

effect curve and thus the bi availability of pilocarpine increase by up to 220 % with respect to a 2 % pilocarpine solution. The bioavailability is defined as the fraction of a drug that is determined with respect to the dose in the measuring compartment, with a direct correlation existing between the concentration and the effect of the drug. The effect of pilocarpine is prolonged by up to 100 % (duration of effect [h]).

TABLE II

Preparation	Duration [h]	AUC [cm^2]	Bioavailability [%]
Pilocarpine 2% Reference	3.5	19.03	100.0
Pilocarpine 2% Nanoparticle 2%	5.5	20.28	205.26
Mucin 4.5% Microparticle 4% Pilo. 2%	7.0	31.53	319.12

Example 3: Loading of Hydrocortisone onto Nanoparticles

Nanoparticles as described in example 1(A) are suspended in water and added to a saturated solution of hydrocortisone in ethanol (13.33 mg/ml). The mixture is ultrafiltrated through a 10 nm filter and the hydrocortisone-adsorbed nanoparticles are retained. The hydrocortisone contained in the filtrate is subsequently spectroscopically determined at 247 nm. The nanoparticles contain 6.81 % of hydrocortisone. The amount of hydrocortisone loaded onto particles with a diameter of 0.8 to 1.5 µm is 4.02 %.

Literature:

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CLAIMS

1. A carrier system for drugs, said carrier system comprising spherical particles with a diameter of less than 1 μm.

2. The carrier system according to claim 1, wherein the spherical particles have a diameter of less than 500 nm.

3. The carrier system according to claim 1, wherein the spherical particles have a diameter of 100 to 300 nm.

4. A carrier system for drugs, said carrier system comprising spherical particles with a diameter of at least 1 nm and less than 1 mm, and at least one bioadhesive polymer.

5. The carrier system according to claim 4, wherein the bioadhesive polymer has a viscosity of 4×10^{-3} to 100×10^{-3} Pas.

6. The carrier system according to claim 4 or 5, wherein the bioadhesive polymer is neutral or anionic.

7. The carrier system according to any of claims 4 to 6, wherein the bioadhesive polymer is selected from the group consisting of polysaccharide, polyacrylate, alginate, polyvinyl alcohol, polyethylene glycol, polyvinyl pyrrolidone and lectine.

8. The carrier system according to any of claims 4 to 7, wherein the bioadhesive polymer is selected from the group consisting of methyl cellulose 400, sodium carboxymethyl cellul se, Carbopol 941, hydroxypropyl methyl cellulose, hyaluronic acid, sodium alginate MV, mucin and polycarbophil.

9. The carrier system according to any of claims 1 to 8, wherein the spherical particles consist of at least one synthetic, semi-synthetic or natural biopolymer.

10. The carrier system according to claim 9, wherein the biopolymer is the protein albumin.

11. A composition consisting of a carrier system according to any of claims 1 to 10 and a drug.

12. The composition according to claim 11, wherein the weight ratio of drug to carrier system is in the range of 100:1 to 1:100.

13. A method for preparing a carrier system according to any of claims 1 to 3, said method comprising at least one of the following method steps in the preparation of the spherical particles:

(A) desolvation of a synthetic, semi-synthetic or natural biopolymer,

(B) thermal denaturation of a synthetic, semi-synthetic or natural biopolymer,

(C) reaction of a synthetic, semi-synthetic or natural biopolymer with a coupling reagent, and/or

(D) reaction of a synthetic, semi-synthetic or natural biopolymer with a compound that contains two or more functional groups.

14. The method according to claim 13, wherein the compound in step (D) is glutaraldehyde.

15. A method for preparing a carrier system according to any of claims 4 to 10, said method comprising at least one of the method steps (A) to (D) according to claim 13, and the further step of mixing the formed spherical particles with at least one bioadhesive polymer.

16. A method for preparing a composition according to claim 11 or 12, said method comprising the step of adding an appropriate drug solution
    (A) during the preparation of the carrier system according to the method of any of claims 13 to 15, and/or
    (B) after the preparation of the carrier system according to the method of any of claims 13 to 15.

17. A method of treatment comprising administering to a patient in need of such a treatment a composition according to claim 11 or 12.

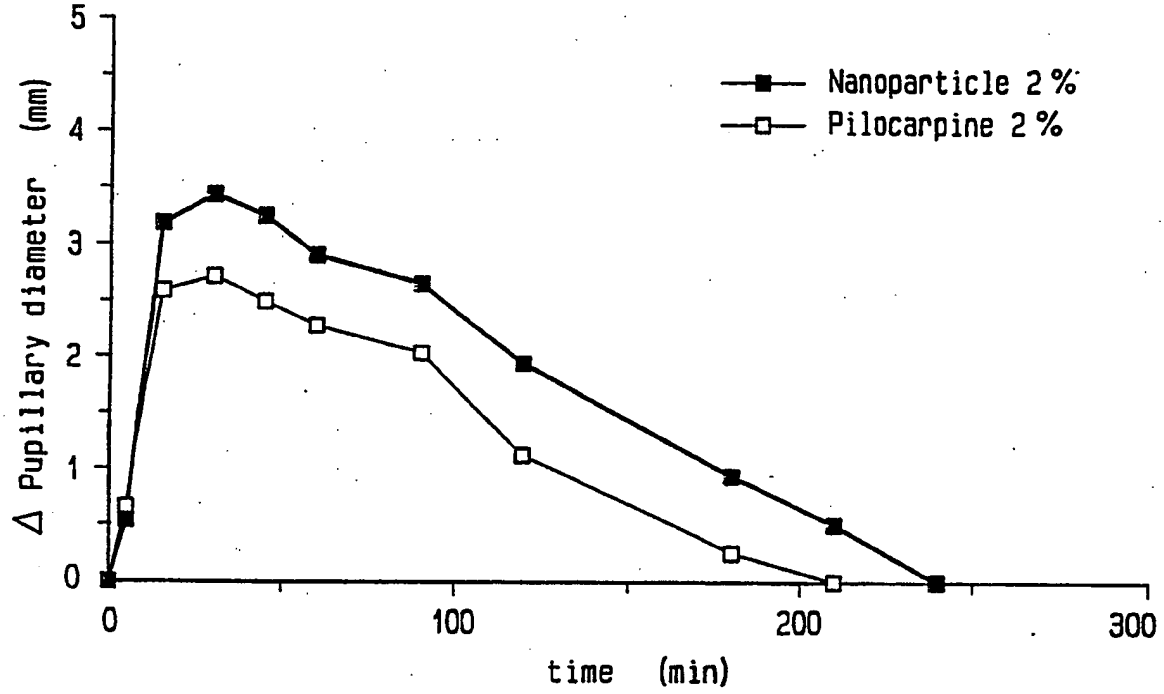
Nanoparticle 2 %
Pilocarpine 2 %
Δ Pupillary diameter (mm)
time (min)
0
1
2
3
4
5
0
100
200
300

Fig. 1

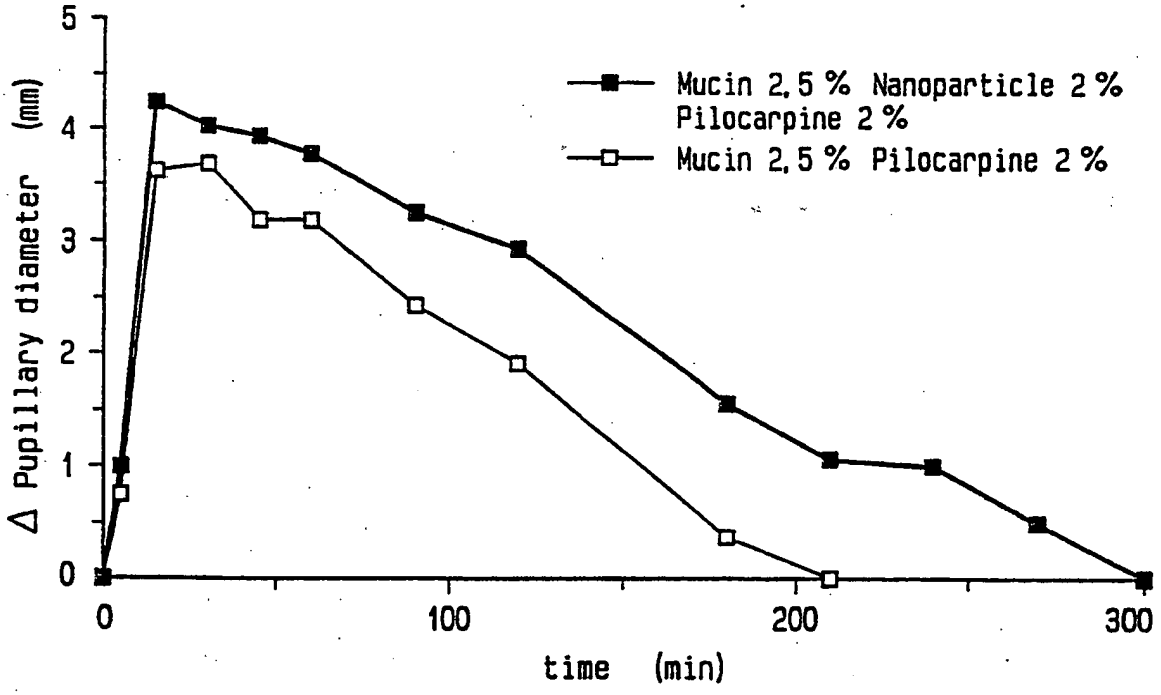
Mucin 2,5 % Nanoparticle 2 %
Pilocarpine 2 %
Mucin 2,5 % Pilocarpine 2 %
Δ Pupillary diameter (mm)
time (min)
0
1
2
3
4
5
0
100
200
300

Fig. 2

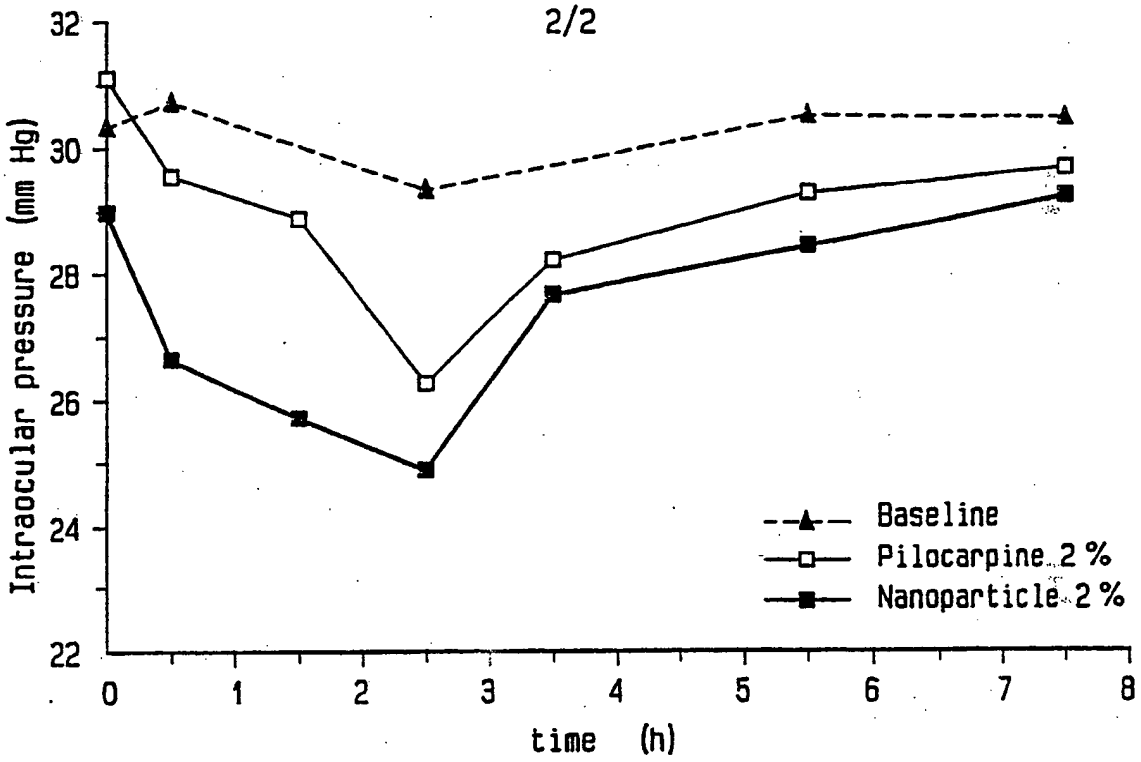
Intraocular pressure (mm Hg)
32
30
28
26
24
22
0
1
2
3
4
5
6
7
8
time (h)
Baseline
Pilocarpine 2 %
Nanoparticle 2 %

Fig.3

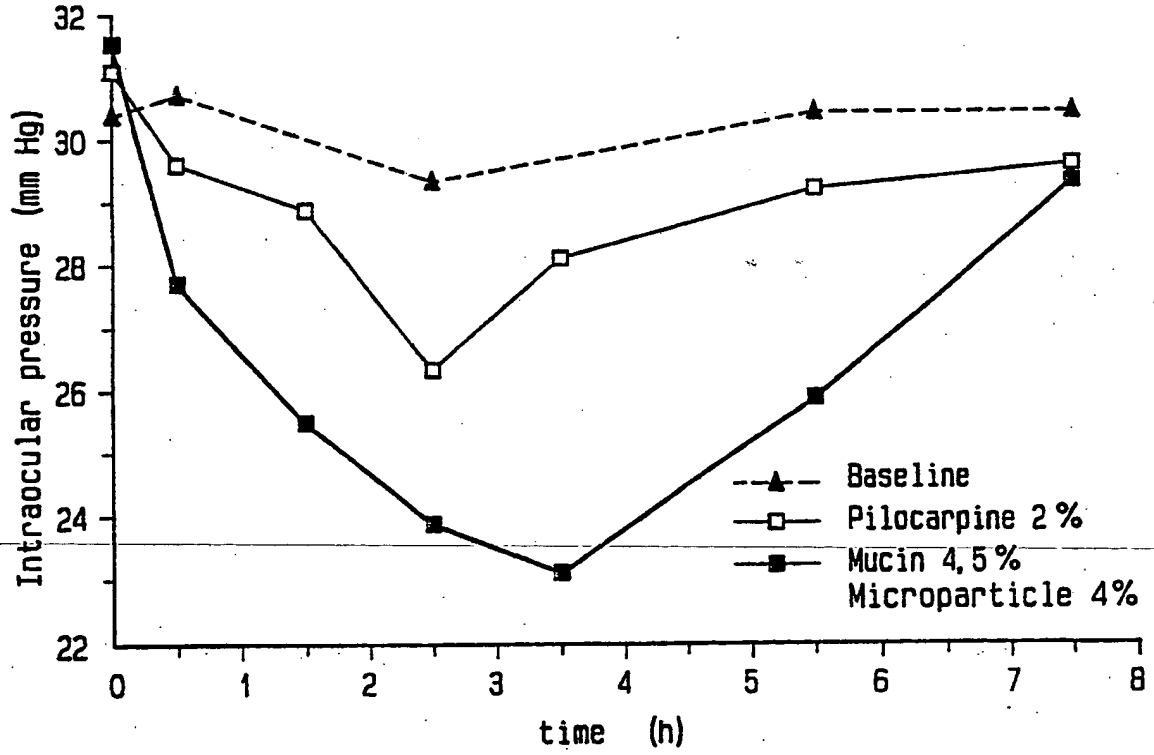
Intraocular pressure (mm Hg)
32
30
28
26
24
22
0
1
2
3
4
5
6
7
8
time (h)
Baseline
Pilocarpine 2 %
Mucin 4,5 %
Microparticle 4%

Fig.4